Standardized laboratory tests with 21 species of temperate and tropical sepsid flies confirm their suitability as bioassays of pharmaceutical residues (ivermectin) in cattle dung

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Standardized Laboratory tests of the livestock parasiticides ivermectin using various temperate and tropical sepsid dung fly species

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Abstract — Veterinary pharmaceuticals excreted in the dung of treated livestock can have strong non-target effects on the dung organism community. We report results of ecotoxicological tests with ivermectin for 21 species of temperate (Europe, North America) and tropical (Asia, Central America) black scavenger flies (Diptera: Sepsidae), using standardized methods developed previously for the yellow dung fly and the face fly. Our study documents large variation in ivermectin sensitivity of more than two orders of magnitude among species and even populations within species: estimated lethal effect concentrations LC50 (at which 50% of the flies died) ranged from 0.05 – 18.55 µg / kg dung fresh weight (equivalent to 0.33 – 132.22 µg / kg dung dry weight). We also show that controlled laboratory tests can – within reasonable limits – be extended to the field or to laboratory settings without climate control, as obtained LC50 were roughly similar. In addition to lethal effects, our study revealed relevant sub-lethal effects at lower ivermectin concentrations in terms of prolonged development, smaller body size and reduced juvenile growth rate. Finally, oviposition choice experiments showed that females generally do not discriminate against dung containing ivermectin residues. We conclude that sepsid flies are well suited test organisms for pharmaceutical residues in the dung of livestock due to their ease and speed of rearing and handling, particularly in the tropics, where high-tech laboratory equipment is often not available.

Keywords—Dung community, Insect, Ecotoxicological test, Oviposition choice

INTRODUCTION

Veterinary pharmaceuticals excreted in the dung of treated livestock can have strong non-target effects on the dung community (e.g. Lumaret et al., 2012; Wall and Strong, 1987), and consequently on the environment in general. Typically some proportion of dung dwellers, primarily beetles and flies, are negatively affected, ultimately impeding the important ecosystem function of breaking down the dung (Floate et al., 2005; Jochmann et al., 2011). Systematic disturbance of the dung community by anthropogenic substances thus raises concerns, to the extent that in the USA, the EU and Japan regulators mandate environmental risk assessments (ERA) for residues of potentially toxic substances excreted in livestock dung (EC, 2009; VICH, 2004; VICH, 2000). This concern is especially true for specific parasiticides such as avermectins (Liebig et al., 2010; Lumaret et al., 2012). As part of such an ERA, standardized tests with non-target organisms have to be performed, usually according to OECD guidelines.

For dung dwellers, single species ecotoxicological laboratory tests recently have been developed for two flies, the yellow dung fly *Scathophaga stercoraria* L. (Diptera: Scathophagidae) and the face fly *Musca autumnalis* L. (Diptera: Muscidae) (Römbke et al. 2010a, 2009). Subsequently, these test protocols were transformed into standardized guidelines by the OECD (OECD, 2010; OECD, 2008). The tests revealed not only lethal but also non-lethal effects in terms of reduced growth, body size and retarded development at lower substance concentrations (Römbke et al., 2009), which are also relevant in the natural situation (e.g. Blanckenhorn, 1998). It is generally clear that any single test species cannot capture, and hence typify, the diversity of sensitivities to any particular toxic substance present in natural communities. In fact, typical ecotoxicological test species, such as the yellow dung fly

74 or the face fly (Römbke et al., 2010a, 2009), are likely to be common, widespread,
75 easy to rear in the laboratory, have a low sensitivity to fluctuations in environmental
76 conditions, and show broad sensitivity towards man-made pollutants in general
77 (otherwise they would have not been selected; Løkke and Van Gestel, 1998;
78 Römbke et al., 2010b). At the moment globally valid OECD standards require the use
79 of the same test species (usually of temperate origin). However, there is some
80 agreement among regulators that in the future regional abiotic (e.g. test conditions
81 such as temperature) and biotic (e.g. species) differences must be taken into account
82 in ERA (e.g. EFSA, 2010; Römbke et al., 2010b). Various test species representing
83 the different biogeographic regions of the world (e.g. tropical vs. temperate) are
84 therefore desirable when assessing the effects of parasiticides on the dung
85 community.

86 The aim of this study was to investigate the effects of a parasiticide on 21
87 species of temperate (Europe, North America) and tropical (Asia, Central America)
88 sepsid dung or black scavenger flies (Diptera: Sepsidae) in standardized
89 ecotoxicological tests. As a model substance we used the parasiticide ivermectin,
90 and we designed our tests like those for other dung flies (Römbke et al., 2010a,
91 2009). Ivermectin is commonly applied to a variety of livestock species worldwide to
92 eliminate parasitic nematodes, but also arthropods like ticks and lice (Floate et al.,
93 2005; Liebig et al., 2010). Sepsid flies are distributed worldwide (Blume, 1985; Pont
94 and Meier, 2002). They are small, locally common, and easy to rear in large groups
95 (not unlike *Drosophila*) on cattle dung. In addition, they have short generation times
96 of ca. 2 weeks (e.g. Blanckenhorn et al., 1998). We were especially interested in
97 whether multiple geographic populations differ in their sensitivity, using mortality
98 (LC50) and non-lethal effects on growth, development and body size as assessment

criteria. We further tested whether four of the temperate species differ in sensitivity when being exposed in parallel under controlled laboratory conditions as well as under naturally variable field conditions to determine whether such tests can also function in uncontrolled, natural settings. Finally, results of oviposition choice experiments for a subset of the species were used to investigate whether sepsid females can discriminate dung contaminated with ivermectin.

1. MATERIAL AND METHODS

2.1 Emergence tests

We followed the methods and standards specified in Römbke et al. (2010a, 2009) and OECD (2008). Tests of 21 sepsid species were performed over a period of 4 years (2008 – 2011) in temporal blocks. Dung used in all tests was originally collected fresh from cattle in the field that had not been treated with parasiticides for at least three months, and was subsequently frozen at -20 °C for at least 4 weeks before being used. The dung was spiked with ivermectin, using technical ivermectin (CAS-No. 70288-86-7) with a purity of 94% ivermectin B1a and 2.8% ivermectin B1b (Merial, Atlanta, GA, USA). Ivermectin was first dissolved in acetone to obtain the desired concentrations by serial dilution. The acetone/ivermectin solution was then thoroughly mixed into cattle dung and kept overnight at room temperature to allow for evaporation of the solvent. Each test comprised 8 treatments: a blank control, an acetone control, and six ivermectin concentrations ranging from 0.21 to 65.7 μg ivermectin / kg dung fresh weight. As the dry matter content of the dung used was determined as 14.03%, these numbers were equivalent to 1.48 – 468.28 μg ivermectin / kg dung dry weight. In a few cases (especially with insensitive species) an extra high concentration was tested in addition (207 μg ivermectin / kg dung fresh

124 weight or 1483 μg ivermectin / kg dung dry weight). No analytical verification was
125 performed, but it is known from literature that ivermectin is highly persistent in dung
126 (e.g. Liebig et al., 2010).

127 All flies used were originally caught wild at the various sites specified in Table
128 1 and kept for multiple generations in our laboratories in Zürich and/or Singapore
129 (see e.g. Blanckenhorn et al., 1998, for rearing methods). For the tests, multiple
130 dishes (size 22 (width) x 44 (length) x 6 (depth) mm^3) filled with a thin layer of fresh
131 dung were put overnight into several population containers per species so that
132 females could lay eggs into them. Ca. 24 h later larvae hatched and could be
133 collected, using a fine brush, from the surface as the dung layer was slowly drying.
134 The experimental units were the same plastic dishes (22 x 44 x 6 mm^3) filled entirely
135 with ca. 6 g (fresh weight) of test dung (5 replicates per treatment). Typically 10 – 15
136 larvae from several holding containers (i.e. mothers) were counted into each
137 experimental dish. Each dish was then transferred into a 50 ml glass tube capped
138 with a paper stopper and incubated in a climate chamber at 21 °C and, additionally
139 for only some populations (see Table 1), at a fully shaded field site outdoors close to
140 the University of Zürich-Irchel.

141 The number of adult flies (of both sexes) emerging from each dish was recorded
142 to document the lethal effect of ivermectin residues in the dung. Sub-lethal effects were
143 additionally assessed by recording the (larva to adult) development times of all
144 emerging flies (always adding one day for preceding egg development), as well as
145 their head width as a practical surrogate for body size. Males and females typically
146 differ in development time and body size, so data were recorded separately for the
147 sexes. Growth rates could then be calculated simply as head width / development
148 time.

Separately for each population and species (i.e. each test listed in Table 1), ivermectin concentrations causing 50% larva-to-adult mortality (LC50) were estimated using probit analysis of logit-transformed emergence proportions against $\log_{10}$(ivermectin concentration), as for binary data sigmoid relationships are expected. Analogous sex-specific linear regressions were employed to assess the effect of $\log_{10}$(ivermectin concentration) on development time, body size and growth rate (untransformed raw values in all cases). The acetone control was set to 0.1 ivermectin equivalents and the blank control to 0.09 for purposes of analysis, because otherwise all concentration values of zero would have been excluded. In the existing OECD guidelines for the larger dung flies *Scathophaga stercoraria* and *Musca autumnalis* validity criteria of 60 – 70% adult emergence are required (OECD 2008). However, due to the lack of experience with sepsid flies, here a test was considered valid if larva-to-adult survival in the combined water and acetone control treatments exceeded 50%, though most species had control mortality rates <30%). In any case, the LC50 values were calculated relative to the control mortality.

2.2 Oviposition choice experiments

For the oviposition site choice experiments, a total of 4 – 14 replicate containers of various sepsid populations/species were each offered a plastic dish (22 x 44 x 6 mm^3) with test dung of each of the above six ivermectin concentrations plus the two blank and acetone controls in a randomized spatial array. Females in each population container were given about 4 h to oviposit, whereupon all eggs laid into each dish were counted. Linear regressions of the square-root-transformed number of eggs laid per dish on $\log_{10}$(ivermectin concentration) were performed to test whether a given species/population significantly avoided or preferred dung with

ivermectin.

2. RESULTS

3.1 Emergence tests

Table 1 lists the estimated Lethal effect Concentrations LC50 (at which 50% of the flies died), plus their (asymmetric) 95% confidence intervals (CI), in terms of fresh dung and dry dung matter. Overall LC50 values varied considerably by more than two orders of magnitude (0.05 – 24.58 µg / kg dung fresh weight, and 0.33 – 175.21 µg / kg dung dry weight). Figure 1 shows exemplary data for one species, *Sepsis monostigma*.

A general linear model (GLM) analyzing only those four species that were reared both in the laboratory and the field (*Sepsis cynipsea, S. fulgens, S. punctum, S. thoracica*) showed no differences in LC50 values between the rearing conditions (interaction test reflecting the (sigmoid) slope: $F_{1,774} = 1.413$, $P = 0.238$), demonstrating that, at least for the lethal effect, our ecotoxicological test is robust against abiotic environmental variation (Table 1). Note that at the same time both development time and body size typically varied, sometimes markedly, between the laboratory and the field (Table 2), an unsurprising result given that temperatures differed strongly and insect life history traits are typically very sensitive to such environmental variation (e.g. Blanckenhorn, 1999).

For those seven temperate species for which we tested multiple populations (*Sepsis cynipsea, S. fulgens, S. neocynipsea, S. orthocnemis, S. punctum, S. thoracica, S. violacea*), an analogous GLM revealed that LC50 values differed not only among species (species by ivermectin concentration interaction: $F_{6,850} = 3.25$, $P = 0.004$) but also among populations within species (population by ivermectin

199 concentration interaction: $F_{12,850} = 2.40$, $P = 0.005$). Variance component analysis
200 revealed approximately as much variance among species as there was among
201 populations within species. Again, this could be expected given that for two species
202 (*S. neocynipsea, S. punctum*) North American and European populations were
203 included, which differ strongly genetically and in their life history (including
204 development time and body size: Table 2).
205 In addition to the above lethal effects, our tests also revealed sub-lethal effects
206 in terms of prolonged development or reduced growth rate and body size (Table 2).
207 Of 41 individual tests, 32 showed a positive linear relationship between development
208 time and $\log_{10}$(ivermectin concentration) (both sexes combined; two-tailed binomial
209 test: $P = 0.004$); of these 18 were significantly positive (and none significantly
210 negative, while all other relationships have to be considered nil), indicating overall
211 longer development as substance concentration increased (Table 2; e.g. Fig. 1b).
212 Similarly, 33 tests showed a negative linear relationship between body size (head
213 width) and $\log_{10}$(ivermectin concentration), of which albeit only 9 were significantly
214 negative (and also 2 significantly positive), indicating overall reduced body size with
215 increasing substance concentration (Table 2; e.g. Fig. 1c). When combining both
216 effects in terms of growth rate (= body size / development time), 35 of 41 tests
217 showed a negative relationship, with 16 significantly negative and only 1 significantly
218 positive (Table 2; e.g. Fig. 1d). Thus, in summary, a reduction of juvenile growth by
219 ivermectin was common but not universal among sepsids, only sometimes being
220 effected by developmental delays, and only sometimes resulting in reduced final
221 body size. All interaction tests were highly significant ($P < 0.001$), indicating strong
222 heterogeneity among species and populations in their life history responses to
223 ivermectin.

224

225 *3.2 Oviposition choice experiments*

226 A total of 21 oviposition tests were performed using often multiple populations
227 of 10 temperate sepsid species (Tab. 3; e.g. Fig. 2). In general, sepsid females did
228 not discriminate among oviposition sites (i.e. miniature dung pats) featuring different
229 (sometimes lethal) ivermectin concentrations, thus regularly subjecting their offspring
230 to detrimental substance doses. In most tests there was no relationship between
231 $\log_{10}$(ivermectin concentration) and the number of eggs deposited (e.g. Fig. 2). Only
232 2 tests showed a significantly positive relationship, if anything indicating preference
233 of ivermectin-contaminated dung (Table 3).

234

235 **3. DISCUSSION**

236 Our study demonstrates that standardized laboratory ecotoxicological tests of
237 the sort developed for the yellow dung fly *Scathophaga stercoraria* and the face fly
238 *Musca autumnalis* (OECD, 2008; Römbke et al., 2010a, 2009) also function well with
239 various sepsid species. All relevant criteria for the selection of ecotoxicological test
240 species such as ecological relevance, practicability in breeding and test performance,
241 general intermediate sensitivity, etc. were fulfilled. However, validity criteria and the
242 expected toxicity range of a reference substance (probably ivermectin) have to be
243 fixed, preferably based on the results of multiple laboratory or ring tests. Our results
244 are applicable in general, as we tested tropical as well as temperate species and
245 populations from Europe, Asia, North and Central America. We also show that such
246 tests can – within reasonable limits – be extended to the field or to laboratory settings
247 without climate control, as the lethal effects (LC50) obtained were roughly the same
248 under both conditions. Crucially, our study revealed considerable variation in

ivermectin sensitivity of at least two orders of magnitude among sepsid species and even populations within species. This indicates that any single test species cannot possibly be representative in terms of assessing toxicity of any substance in the dung community (discussed in more detail below). In addition to lethal effects, our study uncovered relevant sub-lethal effects at lower ivermectin concentrations in terms of reduced growth rates (cf. Römbke et al., 2009, but see Römbke et al., 2010a). Finally, as could be expected a priori and from similar experiments with yellow dung flies (Römbke et al., 2009), ovipositing sepsid females generally do not discriminate against dung containing ivermectin residues. We discuss these findings in more detail below.

A striking result of our study is the 500-fold variation in ivermectin sensitivity of closely related sepsid dung flies. The least sensitive species, *Microsepsis* spp., *S. punctum* and *S. monostigma*, have LC50s comparable to those of the common yellow dung fly (Römbke et al., 2009), which was found to be not very sensitive (LC50 around 20 µg / kg fresh dung), while *Musca autumnalis* proved to be more sensitive (LC50 around 5 µg / kg fresh dung: Römbke et al., 2010a), similar to e.g. *S. fulgens* here. Notably, several sepsids proved to be even more sensitive, with LC50s well below 1 µg / kg fresh dung, among them the most common species around cow dung in central Europe, *S. cynipsea*, and the most common species in North America, *S. neocynipsea*, which are closely related sister species. Therefore, common species do not necessarily have low sensitivities to toxic substances. Large variation in sensitivity to parasiticides also makes choice of test species (which is a central part of the ERA process) particularly delicate. In our opinion the only solution to the problem is to allow use of several test species, or even the dung community as a whole (Floate et al., 2005; Jochmann et al., 2011), for ERA of veterinary

pharmaceuticals, by extending and specifying the higher tiers of the registration process already required for these drugs (VICH, 2004). This should also include the issue of regionalization, which is already discussed in the context of pesticide registration (EFSA, 2010). In general, for ease of rearing and handling, we can highly recommend sepsid flies as test species in this context. In fact, given so much variation among species in sensitivity, one may as well use different species for local tests depending on availability. Thus, in the tropics the locally common *S. lateralis, S. dissimilis or Meroplius fukuhari* could be used, whereas in Europe *S. cynipsea* would be the species of choice. Moreover, as shown here at least regarding the lethal LC50 effects, the test environment is of little concern, allowing tests without expensive climate control or even under field conditions, for example in tropical laboratories.

We emphasize that the concentrations used here are by no means exceptional in the field. Cattle topically treated with ivermectin at the recommended dosage of 500 µg / kg body weight excreted residue concentrations of 205 (3 d post-application) to 30 (12 d post-application) µg / kg fresh weight (Lumaret et al., 2007). When treated with ivermectin injections at the recommended dosage of 200 µg / kg body weight, excreted residues ranged from 200 (3 d post-application) to 10 (28 d post-applications) µg / kg fresh weight (Herd et al., 2006). Similar ranges of ivermectin residues from 1150 (3 d post-application) to 22.8 µg / kg fresh weight (29 d post-application) were found by Suarez et al. (2003).

Fecal residues of veterinary pharmaceuticals can have additional sub-lethal effects on insects breeding in dung, which necessarily influence their performance in the natural habitat (reviewed in Floate et al. (2005) and Jochmann et al. (2011)). For example, smaller flies often have lower reproductive success in the field (e.g. Jann et al., 2000), and longer development times may be detrimental in time-limited

299 situations when the winter is approaching or when the dung pat is drying or being
300 exhausted (e.g. Blanckenhorn, 1998). It is therefore sensible that the OECD
301 Guideline protocol (2008) recommends measuring developmental time as well as
302 morphological traits such as body size or wing deformations of adult flies (cf. Strong
303 and James (1992), but see Floate and Coghlin (2010)) in addition to mortality effects.
304 Such measurements require little additional effort, yet they can be sensitive
305 indicators for the presence of toxic residues.

306 As already discussed for the yellow dung fly (Römbke et al., 2009), oviposition
307 choice experiments indicate that most dung breeding sepsids also cannot perceive
308 even high and lethal ivermectin concentrations in dung. From an evolutionary
309 perspective this is perhaps unsurprising given the short time ivermectin is in use
310 (since 1974). Ovipositing females thus are unable to avoid any dung conditions
311 detrimental to their larvae, even though there are reports of some species being
312 particularly attracted to dung containing parasiticide residues (Floate, 2007).

313 We close by reiterating that sepsid flies are very well suited as test organisms
314 for any toxic residues in the dung of livestock or other large vertebrates, due to their
315 ease and speed of rearing and handling. While the choice of a particular species will
316 be crucial because species vary strongly in sensitivity, use of several local species
317 can offset the arbitrariness of choice to some degree, rendering overall
318 representative results. Sepsids as ecotoxicological test organisms could be
319 particularly useful and economical in the tropics, where high-tech laboratory
320 equipment is often not available.

321

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Figure captions

Figure 1: Exemplary plots for the sub-tropical Chinese species *Sepsis monostigma* (all ± SE): (a) emergence rates for both sexes combined, and sex-specific (males denoted by squares and females by circles) (b) development times, (c) body size (head width), and (d) growth rates as a function of ivermectin concentration plus water & acetone controls. *S. monostigma* was one of the least sensitive species of all, so we had to add an extra high concentration.

Figure 2: Square-root-transformed number of eggs laid by populations of Spanish (squares and solid regression line) and Italian (circles and broken line) *Sepsis thoracica* females into dishes containing dung spiked with various ivermectin concentrations, plus water & acetone controls, in laboratory oviposition choice experiments.

Table captions

Table 1: Proportion of flies emerged from the control treatments (water and acetone) for all 47 tests using 21 sepsid species, plus the estimated lethal concentration at which 50% of the flies died (LC50) with their 95% confidence limits in terms of fresh and dry dung (species averages with SD in italics).

Table 2: Mean ± SE development time and body size for male and female sepsids emerged from the experiment (N = total number of individuals) for all 47 tests using 21 species. The last three columns give the correlation coefficient, for both sexes combined, between the life history trait and log10(ivermectin concentration). Significant correlations are in bold.

442 **Table 3:** Correlation coefficient between the square-root-transformed number of eggs
443 laid by populations of females into dishes with 6 concentrations of ivermectin plus
444 two controls and log10 (ivermectin concentration) for each oviposition choice
445 experiment using a total of 21 populaions of 10 sepsid species (N = number of
446 population cage replicates). A negative correlation indicates avoidance of higher
447 ivermectin concentration, a positive correlation preference thereof. Significant
448 correlations are in bold.

Tables 1-3

Table 1: Proportion of flies emerged from the control treatments (water and acetone) for all 47 tests using 21 sepsid species, plus the estimated lethal concentration at which 50% of the flies died (LC50) with their 95% confidence limits in terms of wet and dry dung (species averages with SD in italics).

Genus	species	population provenance	latitude	longitude	altitude	lab/field	p(emerged)	**LC50 (wet)**	CI95%l	CI95%h	**LC50 (dry)**	CI95%l	CI95%h
Archisepsis	*armata*	Costa Rica: San Jose	9.94	84.05	1208	Lab	0.908	***6.201***	3.577	12.301	***44.198***	25.496	87.679
Archisepsis	*diversiformis*	Costa Rica: San Jose	9.94	84.05	1208	Lab	0.81	***1.923***	1.102	3.541	***13.704***	7.856	25.239
Dicranosepsis	*emiliae*	Vietnam: Tam Dao	21.52	105.55	1000	Lab	0.587	***0.241***	0.133	0.390	***1.720***	0.949	2.777
Microsepsis	*armillata*	Costa Rica: San Jose	9.94	84.05	1208	Lab	0.810	***24.582***	7.252	209.172	***175.209***	51.692	1490.890
Microsepsis	*mitis*	Costa Rica: San Jose	9.94	84.05	1208	Lab	0.811	***24.314***	9.647	96.884	***173.299***	68.760	690.546
Meroplius	*fukuhari*	China: Zhongmu	34.71	113.97	79	Lab	0.557	***0.138***	0.066	0.237	***24.314***	9.647	96.884
Saltella	*sphondylii*	CH: Zürich	47.38	8.68	536	Lab	0.635	***0.199***	0.137	0.274	***1.418***	0.976	1.953
Sepsis	*cynipsea*	A: Vienna	48.20	16.37	187	Lab	0.690	**0.610**	0.357	1.020	**4.348**	2.545	7.270
		CH: Zürich	47.38	8.68	536	Field	0.760	**0.364**	0.266	0.505	**2.594**	1.896	3.599
						Lab	0.820	**0.491**	0.338	0.742	**3.500**	2.409	5.289
		I: Umbria	43.15	12.10	403	Lab	0.533	**0.080**	0.037	0.130	**0.570**	0.264	0.927
		S: Uppsala	59.85	17.63	16	Field	0.650	**0.163**	0.129	0.203	**1.162**	0.919	1.447
						Lab	0.659	**0.199**	0.161	0.246	**1.418**	1.148	1.753
		S: Nyköping	58.67	16.94	10	Lab	0.569	**0.284**	0.175	0.434	**2.024**	1.247	3.093
								0.313	***0.188***		***2.231***	***1.343***	
Sepsis	*dissimilis*	Brunei	4.94	114.95	1	Lab	0.575	***0.107***	0.055	0.172	***0.763***	0.392	1.226
Sepsis	*duplicata*	CH: Zürich	47.38	8.68	536	Lab	0.530	***0.090***	0.052	0.131	***0.641***	0.371	0.934
Sepsis	*flavimana*	CH: Zürich	47.38	8.68	536	Lab	0.580	***0.047***	0.005	0.138	***0.335***	0.036	0.984
Sepsis	*fulgens*	A: Vienna	48.20	16.37	187	Lab	0.781	**5.684**	2.232	23.625	**40.513**	15.909	168.389
		E: Sierra Nevada	37.20	-3.20	1290	Field	0.663	**0.886**	0.477	1.610	**6.315**	3.400	11.475
						Lab	0.581	**0.902**	0.438	1.763	**6.429**	3.122	12.566
		Est: Tartu	58.14	26.91	81	Field	0.748	**1.265**	0.846	1.913	**9.016**	6.030	13.635
						Lab	0.754	**1.209**	0.721	2.007	**8.617**	5.139	14.305
		I: Calabria	40.13	15.18	5	Lab	0.880	**5.567**	3.230	11.006	**39.679**	23.022	78.446
								2.586	***2.360***		***18.428***	***16.822***	
Sepsis	*lateralis*	IND: Sulawesi	1.45	124.84	43	Lab	0.626	**0.804**	0.202	2.880	**5.731**	1.440	20.527
Sepsis	*monostigma*	CHN: Zhongmu	34.71	113.97	79	Lab	0.845	***11.438***	5.166	33.596	***81.525***	36.821	239.458
Sepsis	*neocynipsea*	CH: Zürich	47.38	8.68	536	Lab	0.703	**0.232**	0.190	0.286	**1.654**	1.354	2.038
		I: Umbria	43.15	12.10	403	Lab	0.540	**0.230**	0.144	0.344	**1.639**	1.026	2.452
		AZ: Tucson	32.22	-110.92	757	Lab	0.724	**1.572**	0.795	3.512	**11.205**	5.666	25.032
		IL: Chicago	41.80	-87.65	170	Lab	0.695	**0.733**	0.322	1.686	**5.225**	2.295	12.017
								0.692	***0.633***		***4.931***	***4.510***	
Sepsis	*orthocnemis*	A: Vienna	48.20	16.37	187	Lab	0.875	**9.888**	3.720	51.563	**70.478**	26.515	367.520
		CH: Zürich	47.38	8.68	536	Lab	0.737	**1.090**	0.694	1.739	**7.769**	4.947	12.395
								5.489	***6.221***		***39.123***	***44.342***	
Sepsis	*punctum*	CH: Zürich	47.38	8.68	536	Field	0.777	**1.659**	0.590	5.809	**11.825**	4.205	41.404
						Lab	0.699	**17.423**	5.451	132.141	**124.184**	38.852	941.846
		D: Berlin	52.52	13.40	41	Field	0.795	**1.988**	1.189	3.563	**14.170**	8.475	25.396
						Lab	0.794	**1.995**	1.216	3.505	**14.220**	8.667	24.982
		GA: Athens	33.96	-83.38	228	Lab	0.829	**18.550**	5.435	190.733	**132.217**	38.738	1359.465
		NY: New York	40.78	-73.97	47	Lab	0.760	**4.244**	1.541	16.350	**30.249**	10.984	116.536
								7.643	***8.073***		***54.477***	***57.540***	
Sepsis	*secunda*	NC: Raleigh	35.77	-78.63	96	Lab	0.710	***1.333***	0.568	3.598	***9.501***	4.048	25.645
Sepsis	*thoracica*	A: Vienna	48.20	16.37	187	Lab	0.579	**0.641**	0.249	1.602	**4.569**	1.775	11.418
		E: Sierra Nevada	37.20	-3.20	1290	Field	0.580	**0.195**	0.117	0.296	**1.390**	0.834	2.110
						Lab	0.535	**0.089**	0.053	0.129	**0.634**	0.378	0.919
		I: Calabria	40.13	15.18	5	Field	0.821	**0.351**	0.303	0.410	**2.502**	2.160	2.922
						Lab	0.687	**0.311**	0.241	0.400	**2.217**	1.718	2.851
		I: Umbria	43.15	12.10	403	Lab	0.713	**0.558**	0.393	0.786	**3.977**	2.801	5.602
								0.358	***0.210***		***2.548***	***1.499***	
Sepsis	*violacea*	A: Vienna	48.20	16.37	187	Lab	0.550	**0.457**	0.117	1.149	**3.257**	0.834	8.190
			58.14	26.91	81	Lab	0.817	**1.318**	0.435	3.716	**9.394**	3.100	26.486
								0.888	***0.609***		***6.326***	***4.339***	
Themira	*minor*	CA: Monterey	36.6	121.89	100	Lab	0.766	***1.255***	0.707	2.241	***8.946***	5.042	15.973

Table 2: Mean ± SE development time and body size for male and female sepsids emerged from the experiment (N = total number of individuals) for all 47 tests using 21 species. The last three columns give the correlation coefficient, for both sexes combined, between the life history trait and log10(ivermectin concentration). Significant correlations are in bold.

Genus	species	population	lab/field	time	Nm	Nf	male development time ± SD	female development time ± SD	male head width ± SD	female head width ± SD	development effect	size effect	grwoth effect
Archisepsis	*armata*	Costa Rica: San Jose	Lab	Aug 11	129	136	17.07 ± 1.241	17.23 ± 1.153	1.15 ± 0.033	1.25 ± 0.041	**0.428**	0.117	**-0.318**
Archisepsis	*diversiformis*	Costa Rica: San Jose	Lab	Aug 11	113	104	20.28 ± 0.885	20.02 ± 1.367	1.12 ± 0.02	1.16 ± 0.027	**0.358**	-0.095	**-0.340**
Dicranosepsis	*emiliae*	Vietnam: Tam Dao	Lab	Aug 11	60	74	17.56 ± 0.981	17.54 ± 0.775	0.83 ± 0.029	0.90 ± 0.03	0.175	-0.270	-0.286
Microsepsis	*armillata*	Costa Rica: San Jose	Lab	Aug 11	146	148	15.34 ± 0.786	15.30 ± 1.199	0.81 ± 0.039	0.85 ± 0.04	0.176	**-0.502**	**-0.412**
Microsepsis	*mitis*	Costa Rica: San Jose	Lab	Aug 11	156	156	14.24 ± 0.813	14.03 ± 0.76	0.76 ± 0.034	0.79 ± 0.035	0.065	**-0.510**	-0.077
Meroplius	*fukuhari*	CHN: Zhongmu	Lab	Nov 09	66	62	17.45 ± 0.685	17.13 ± 0.689	0.96 ± 0.041	1.00 ± 0.064	0.127	**-0.325**	**-0.419**
Saltella	*sphondylii*	CH: Zürich	Lab	Oct 09	65	80	20.11 ± 0.991	19.67 ± 0.685	1.08 ± 0.061	1.11 ± 0.060	0.074	-0.126	-0.063
Sepsis	*cynipsea*	A: Vienna	Lab	May 09	102	84	14.40 ± 0.389	14.28 ± 0.616	0.97 ± 0.033	1.02 ± 0.030	**0.371**	0.075	-0.155
		CH: Zürich	Field	July 08	71	47	10.40 ± 0.460	10.38 ± 0.514	1.02 ± 0.037	1.09 ± 0.025	0.009	**-0.378**	-0.100
		CH: Zürich	Lab	July 08	70	75	12.26 ± 0.358	12.31 ± 0.401	1.02 ± 0.022	1.09 ± 0.028	-0.075	-0.154	-0.136
		I: Umbria	Lab	Oct 09	57	53	14.95 ± 0.438	14.48 ± 0.710	0.99 ± 0.041	1.06 ± 0.051	-0.266	**-0.670**	-0.241
		S: Uppsala	Field	Sep 08	67	54	27.56 ± 0.647	27.43 ± 0.546	1.01 ± 0.029	1.08 ± 0.017	-0.069	-0.061	0.007
		S: Uppsala	Lab	Sep 08	73	63	12.74 ± 0.373	12.75 ± 0.350	0.99 ± 0.024	1.07 ± 0.020	**0.414**	0.177	-0.223
		S: Nyköping	Lab	Oct 09	76	73	13.68 ± 0.622	13.65 ± 0.521	0.99 ± 0.019	1.07 ± 0.023	-0.114	**0.346**	0.260
Sepsis	*dissimilis*	Brunei	Lab	Nov 09	52	53	17.76 ± 0.586	17.54 ± 0.445	0.74 ± 0.022	0.75 ± 0.013	**0.278**	-0.039	-0.136
Sepsis	*duplicata*	CH: Zürich	Lab	Oct 09	60	69	21.02 ± 0.942	20.61 ± 0.735	0.74 ± 0.015	0.83 ± 0.020	0.109	0.184	-0.013
Sepsis	*flavimana*	CH: Zürich	Lab	May 09	50	70	21.60 ± 0.515	22.03 ± 0.679	0.91 ± 0.039	0.99 ± 0.032	**0.485**	0.119	-0.174
Sepsis	*fulgens*	A: Vienna	Lab	May 09	75	72	15.50 ± 0.470	15.77 ± 0.538	0.99 ± 0.027	1.03 ± 0.030	0.254	-0.073	-0.219
		E. Sierra Nevada	Field	Sep 08	156	151	23.67 ± 1.240	23.79 ± 1.431	0.97 ± 0.022	1.02 ± 0.023	0.038	-0.075	-0.070
		E. Sierra Nevada	Lab	Sep 08	158	160	17.34 ± 0.610	17.41 ± 0.643	0.94 ± 0.019	0.99 ± 0.025	**0.759**	-0.130	**-0.696**
		Est: Tartu	Field	May 09	160	196	18.10 ± 1.121	18.62 ± 1.167	0.97 ± 0.044	1.02 ± 0.036	-0.018	-0.108	0.001
		Est: Tartu	Lab	May 09	184	170	16.08 ± 1.034	16.17 ± 1.056	0.97 ± 0.021	1.02 ± 0.028	**0.425**	-0.169	**-0.426**
		I: Calabria	Lab	Oct 09	136	127	17.59 ± 0.524	17.93 ± 0.693	0.99 ± 0.018	1.05 ± 0.021	**0.588**	-0.214	**-0.528**
Sepsis	*lateralis*	IND: Sulawesi	Lab	May 09	81	86	16.87 ± 0.716	16.19 ± 0.854	1.08 ± 0.050	1.05 ± 0.035	-0.156	0.017	0.005
Sepsis	*monostigma*	CHN: Zhongmu	Lab	May 09	99	95	14.81 ± 0.530	14.81 ± 0.664	1.03 ± 0.030	1.10 ± 0.026	**0.343**	**-0.438**	**-0.459**
Sepsis	*neocynipsea*	CH: Zürich	Lab	July 08	66	64	17.14 ± 0.409	16.91 ± 0.422	1.12 ± 0.020	1.17 ± 0.028	0.173	0.008	-0.089
		I: Umbria	Lab	Oct 09	64	55	16.90 ± 0.590	16.98 ± 0.529	1.09 ± 0.040	1.12 ± 0.034	0.008	-0.048	-0.040
		AZ: Tucson	Lab	May 09	100	113	14.78 ± 0.607	13.95 ± 0.454	1.15 ± 0.053	1.11 ± 0.038	**0.495**	-0.245	**-0.477**
		IL: Chicago	Lab	May 09	69	93	14.64 ± 0.462	14.04 ± 0.566	1.12 ± 0.041	1.08 ± 0.035	0.259	-0.066	-0.230
Sepsis	*orthocnemis*	A: Vienna	Lab	May 09	78	161	18.03 ± 0.313	18.04 ± 0.208	0.93 ± 0.017	0.99 ± 0.018	0.150	**0.549**	**0.371**
		CH: Zürich	Lab	July 08	92	115	18.38 ± 0.261	18.53 ± 0.550	0.95 ± 0.014	0.99 ± 0.016	**0.474**	-0.196	**-0.443**
Sepsis	*punctum*	CH: Zürich	Field	May 09	52	68	21.08 ± 0.925	19.31 ± 0.363	1.23 ± 0.038	1.15 ± 0.030	-0.124	-0.166	-0.206
		CH: Zürich	Lab	May 09	111	93	16.56 ± 0.617	15.22 ± 0.417	1.30 ± 0.031	1.22 ± 0.045	0.247	-0.209	**-0.403**
		D: Berlin	Field	Sep 08	173	159	26.11 ± 2.056	23.34 ± 1.577	1.30 ± 0.033	1.20 ± 0.030	0.057	**-0.471**	-0.118
		D: Berlin	Lab	Sep 08	156	201	16.09 ± 0.505	14.91 ± 0.614	1.29 ± 0.035	1.21 ± 0.025	**0.415**	**-0.492**	**-0.517**
		GA: Athens	Lab	May 09	130	132	14.24 ± 0.401	13.83 ± 0.509	1.05 ± 0.026	1.08 ± 0.024	**0.299**	-0.058	**-0.285**
		NY: New York	Lab	May 09	101	95	13.81 ± 0.639	13.65 ± 0.846	1.04 ± 0.031	1.07 ± 0.041	**0.511**	-0.252	**-0.269**
Sepsis	*secunda*	NC: Raleigh	Lab	May 09	103	113	22.45 ± 0.606	22.71 ± 1.018	0.79 ± 0.032	0.86 ± 0.040	**0.517**	-0.247	**-0.492**
Sepsis	*thoracica*	A: Vienna	Lab	May 09	83	76	12.04 ± 0.168	11.28 ± 0.380	1.11 ± 0.036	1.03 ± 0.022	**0.497**	**-0.336**	**-0.520**
		E. Sierra Nevada	Field	Sep 08	60	57	21.57 ± 1.836	18.03 ± 1.589	1.16 ± 0.027	1.07 ± 0.015	-0.140	-0.277	0.078
		E. Sierra Nevada	Lab	Sep 08	58	51	12.86 ± 0.431	11.91 ± 0.702	1.13 ± 0.029	1.00 ± 0.066	0.249	-0.202	-0.328
		I: Calabria	Field	July 08	147	134	10.91 ± 1.589	10.14 ± 1.172	1.17 ± 0.052	1.06 ± 0.037	-0.002	-0.228	-0.051
		I: Calabria	Lab	July 08	135	140	12.63 ± 0.64	11.52 ± 0.695	1.16 ± 0.058	1.05 ± 0.053	0.235	-0.079	-0.172
		I: Umbria	Lab	Oct 09	65	63	14.43 ± 0.342	13.44 ± 0.345	1.13 ± 0.043	1.06 ± 0.023	**0.524**	**-0.287**	**-0.473**
Sepsis	*violacea*	A: Vienna	Lab	Oct 09	73	72	21.30 ± 1.106	21.32 ± 0.922	1.02 ± 0.028	1.09 ± 0.028	**0.552**	-0.226	**-0.509**
		Est: Tartu	Lab	Oct 09	66	71	20.72 ± 1.671	20.45 ± 1.028	1.04 ± 0.034	1.10 ± 0.031	**0.481**	**-0.387**	**-0.454**
Themira	*minor*	CA: Monterey	lab	Aug 11	109	101	11.38 ± 0.43	11.28 ± 0.436	0.89 ± 0.037	0.92 ± 0.037	-0.179	-0.097	0.040
											0.221	***-0.144***	***-0.223*** Mean
											0.074	0.064	0.066 95%CI

Table 3: Correlation coefficient between the square-root-transformed number of eggs laid by populations of females into dishes with 6 concentrations of ivermectin plus two controls and log10 (ivermectin concentration) for each oviposition choice experiment using a total of 21 populaions of 10 sepsid species (N = number of population cage replicates). A negative correlation indicates avoidance of higher ivermectin concentration, a positive correlation preference thereof. Significant correlations are in bold.

Species	**population**	**r**	N
Saltella sphondylii	CH	**0.543**	4
Sepsis cynipsea	A	0.049	5
	CH	0.112	8
	S	0.163	9
Sepsis duplicata	CH	-0.039	6
Sepsis flavimana	CH	0.064	6
Sepsis fulgens	E	**0.375**	7
	EST	0.230	7
	I	-0.004	5
Sepsis neocynipsea	I	0.030	5
	IL	0.125	5
Sepsis orthocnemis	A	-0.115	5
	CH	0.127	4
Sepsis punctum	A	-0.170	4
	CH	0.275	5
	D	-0.005	14
	GA	-0.082	5
Sepsis thoracica	E	0.071	4
	I	0.177	10
Sepsis violacea	A	0.119	5
	EST	-0.176	4
Mean		***0.089***	

Figure 1

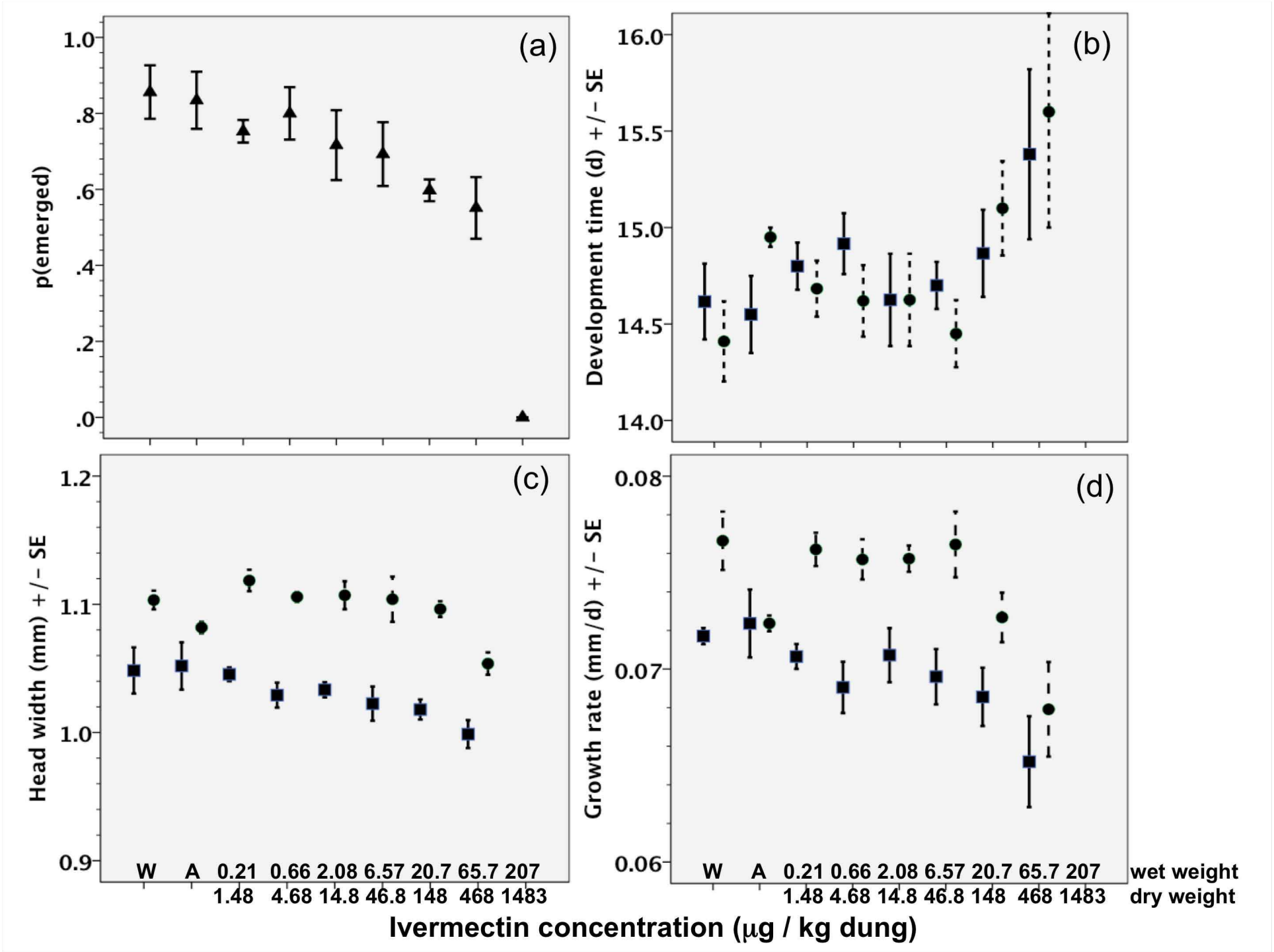
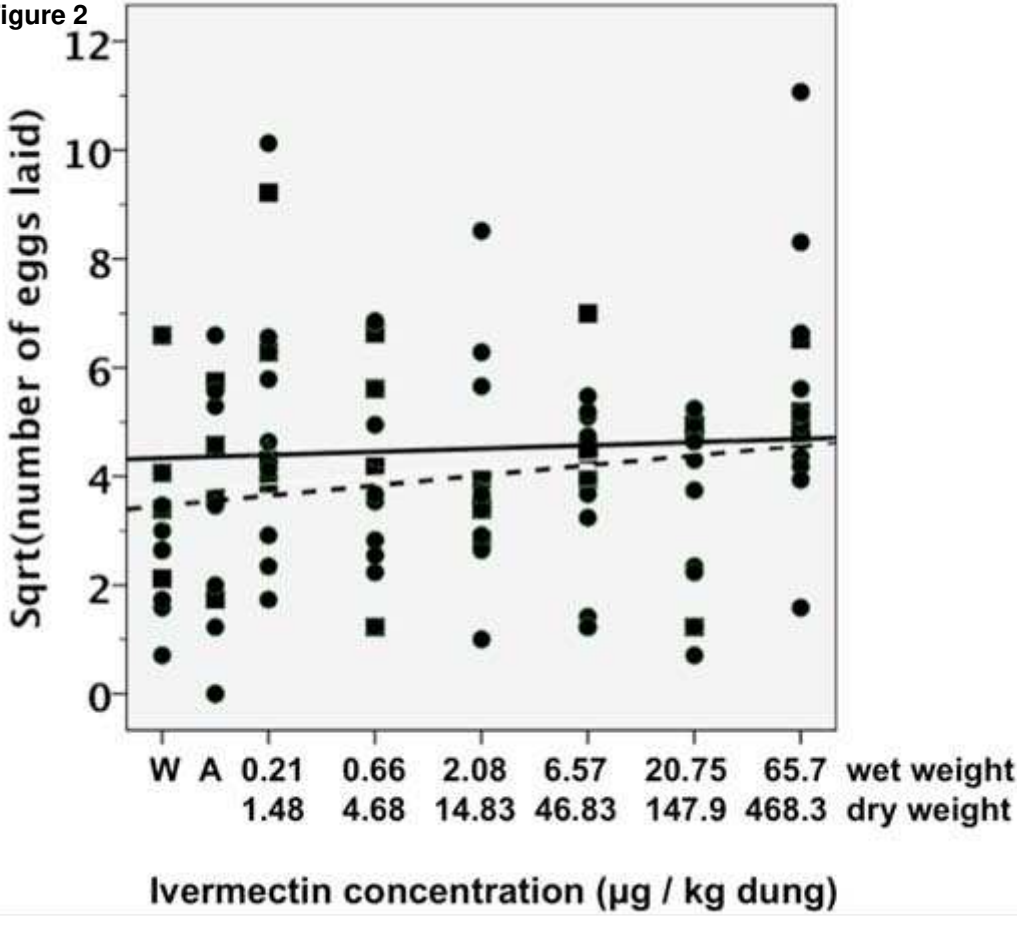

(a)
p(emerged)
1.0
.8
.6
.4
.2
.0
(b)
Development time (d) +/- SE
16.0
15.5
15.0
14.5
14.0
(c)
Head width (mm) +/- SE
1.2
1.1
1.0
0.9
(d)
Growth rate (mm/d) +/- SE
0.08
0.07
0.06
W A 0.21 0.66 2.08 6.57 20.7 65.7 207
1.48 4.68 14.8 46.8 148 468 1483
wet weight
dry weight
Ivermectin concentration (μg / kg dung)

Figure 2


Sqrt(number of eggs laid)
12
10
8
6
4
2
0
W A 0.21 0.66 2.08 6.57 20.75 65.7 wet weight
1.48 4.68 14.83 46.83 147.9 468.3 dry weight
Ivermectin concentration (µg / kg dung)